1-1-1971

Regional Distribution of 5-Methyltetrahydrofolate Dependent Dopamine-N-Methyl Transferase in Rat Brain and Its Presence in Human Brain

Douglas A. Berv
Yale University

REGIONAL DISTRIBUTION OF 5-METHYLTETRAHYDROFOLATE DEPENDENT DOPAMINE-N-METHYL TRANSFERASE IN RAT BRAIN AND ITS PRESENCE IN HUMAN BRAIN

DOUGLAS A. BERY

1974

YALE

MEDICAL LIBRARY

Regional Distribution of 5-Methyltetrahydrofolate Dependent Dopamine-N-Methyl Transferase in Rat Brain and Its Presence in Human Brain

Douglas A. Berv

A thesis presented to the Department of Psychiatry Yale University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine

1974

Acknowledgements

I wish to thank Dr. Robert Roth for his guidance at every stage of the thesis. His knowledge, patience, and encouragement were instrumental in my discovering the joys of research.

In addition, I thank Dr. Judy Walters, Karen Brady, Dr. Jim Bennett, Dr. Dorothy Gallager, Betty Herr, Jan Abele, Sue Smith, and Ken Giddings for their help; it was a pleasure to work with them in the lab. My brother Ken gave me both encouragement and technical advice. I am also grateful to Dr. George Aghajanian, Dr. Elias Manuelidis, Dr. Robert Byck, Ann Morrison, Elaine Shrewsbury, Arlene Cashmore, Alvin Prusoff, and Wilma Korevaar.

Table of Contents

Abstract

Since the introduction of the hypothesis that abnormal transmethylation of biogenic amines may be associated with mental illness, transmethylation processes have been shown to be important in the production of biologically active amine derivatives. Laduron has shown that dopamine can be N-methylated with an enzyme from rat brain that requires 5-methyltetrahydrofolic acid (5MTHF) as a methyl donor. This study shows that the methylation reaction which produces epinine from dopamine is greatest with supernatant from homogenized caudate lobe as compared to the cerebellum, hippocampus, cortex, and raphe nucleus: the brains of male rats were dissected and homogenized, the supernatant was assayed for 5MTHF dependent dopamine-N-methyl transferase activity by incubation with dopamine and C^{14}-5MTHF, separation of the the catecholamines with alumina columns, counting radioactivity with a scintillation counter, and identification of epinine with Amberlite column chromatography. The medial forebrain bundle (DA containing neurons) and the raphe nucleus (5HT containing neurons) were lesioned. There was no significant change in enzyme activity in the caudate after these lesions. Human caudate tissue was assayed and found to have significant enzyme activity.

Introduction

In 1884 J.L.W. Thudichum wrote:

> Many forms of insanity are unquestionably the external manifestations of the effects upon the brain-substance of poisons fermented within the body, just as the mental aberrations accompanying chronic alcoholic intoxication are the accumulated effects of a relatively simple poison fermented outside the body. These poisons we shall, I have no doubt, be able to isolate after we know the normal chemistry to its uttermost detail. And then will come in their turn the crowning discoveries to which all our efforts must ultimately be directed, namely, the discoveries of the antidotes to the poisons, and to the fermenting causes and processes which produce them. (1)

This hypothesis that mental illness results from abnormal metabolism in the brain received renewed interest in 1952 when Osmond and Smythies (2) discussed that it had been known for a long time that the symptoms of mescaline intoxication and schizophrenia were very similar and, in addition, that mescaline's chemical structure was close to that of adrenaline. A biochemical note by Harley-Mason published in their paper said:

> It is extremely probable that the final stage in the biogenesis of adrenealine is a transmethylation of noradrenaline, the methyl group arising from methionine or choline. It is just possible that a pathological disordering of its transmethylation mechanism might lead to methylation of one or both of its phenolic hydroxyl groups instead of its amino group...Methylation of phenolic hydroxyl groups in the animal body is of rare occurrence, but a significant case has been reported recently....It is particularly interesting to note that out of a series of phenylethylamine derivatives tested by Noteboom, 3,4-dimethoxyphenylethylamine was the most potent in producing catatonia in animals....

Thus began the work that has shown transmethylation processes to be important in the production of biologically active amine compounds. Most of this research has been concerned with transmethylation enzymes which use S-adenosyl-methionine (SAM) as a carbon donor. Laduron (3) has recently reported an enzyme in rat brain which can convert dopamine (DA) to N-methyl dopamine (epinine) using 5-methyl tetrahydrofolic acid (5MTHF) as the carbon donor. This thesis is:

1. a study of the regional distribution of the 5MTHF dependent dopamine-N-methyl transferase in rat brain.
2. a study of the effect on this enzyme of the destruction of DA and serotonin (5HT) containing cells in the brain.
3. a demonstration of the presence of the enzyme in post mortem human caudate tissue.

Transmethylation Enzymes

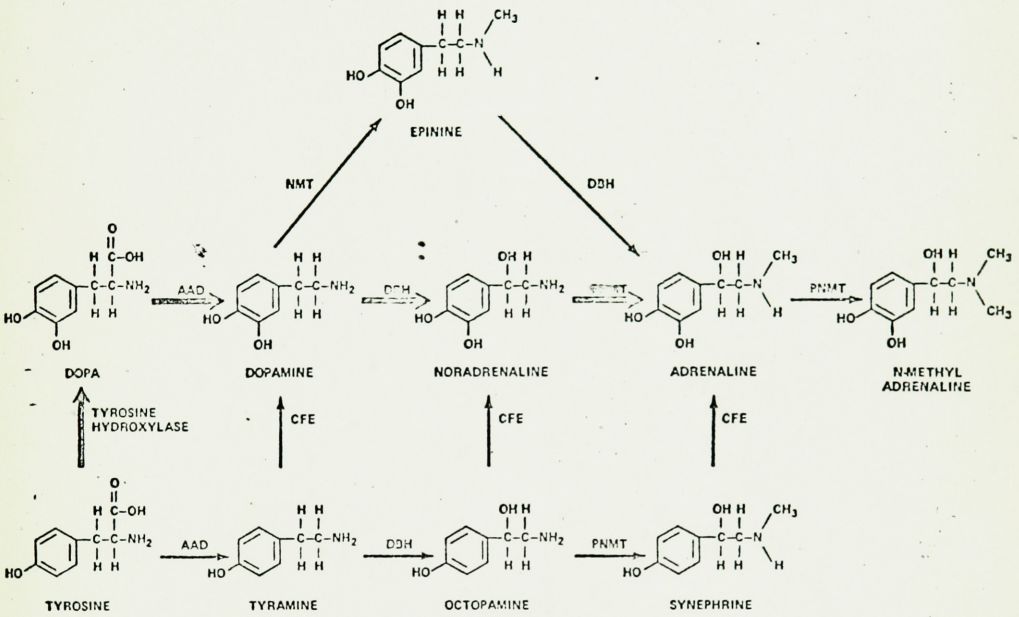
After the proposal of the Harley-Mason hypothesis it was noted (4) that LSD and 5HT showed a pharmacological antagonism, and it was suggested that serotonin might be N- and O- methylated to form various psychotomimetic derivatives. In 1957 Axelrod (5) reported that his search for an enzyme that would inactivate noradrenaline and adrenaline had led to the discovery of the O-methylation of adrenaline to metanephrine. The enzyme, catechol-O-methyltransferase (COMT), was purified (6) and found to require SAM and Mg^{+2}. It was non-specific, being capable of O-methylating all catechols.

The discovery of COMT was followed by the discovery of a whole series of methyl transferase enzymes which are listed in Table I (7). Some of these enzymes are capable of making psychotomimetic compounds as proposed by Harley-Mason. Axelrod (8,9) found a non-specific N-methyl transferase highly localized to rabbit lung that used SAM as a carbon donor and was able to N-methylate 5HT to the psychotomimetic products bufotenine and N-N-dimethyltryptamine (DMT). This enzyme also N-methylates phenylethanolamine derivatives such as mescaline and dopamine. This enzyme was found almost entirely in rabbit lung, an unlikely place for the action of psychotomimetic drugs. Phenylethanolamine N-methyl transferase (PNMT) was found in mammalian adrenal medulla and was capable of transferring a methyl group to a variety of phenylethanolamine derivatives (10). Mandell and Morgan (11) found an enzyme in chick, sheep, and human brain that N-dimethylated tryptamine to form DMT; and Saavedra and Axelrod (12) showed that rat brain was capable of forming DMT in vivo.

PNMT participates in the major accepted pathway for the biosynthesis of the catecholamines shown in Figure 1 (13). An alternate pathway to adrenaline which bypasses noradrenaline was first proposed by Hallé in 1906 (13). After DA is N-methylated to epinine it is β-hydroxylated to adrenaline. It was confirmed that dopamine-β-hydroxylase could do this by Bridgers and Kaufman (14). Nothing is known about the

Methyltransferase	Substrate	Product	Physiological Activity of Product	Localization of Enzyme
Catechol-O	Adrenaline and other catechols, dihydroxyindoles	Metanephrine and other O-methylated metabolites	Decreased	Widespread
Hydroxyindole-O	N-Acetylserotonin, 5-hydroxyindoles	Melatonin and other methoxyindoles	Increased	Pineal body
Histamine-N	Histamine	Methylhistamine	Decreased	Widespread, highest in brain
Phenylethanolamine	Noradrenaline and other phenylethanolamines	Adrenaline and other N-methylated phenylethanolamines	Increased	Adrenal medulla
Lung enzyme	Serotonin, phenylethylamines, catecholamines, normorphine, nornicotine, aniline, purines	N,N-Dimethylserotonin, N-methylated phenylethylamines, N-methylated catecholamines, morphine, nicotine, methylated purines	Increased	Lung
Methanol-forming enzyme	Water	Methanol	Increased	Pituitary

Table I. Methyl Transferase Enzymes and their Properties(7)



Biosynthesis of catecholamines. DBH—dopamine-β-hydroxylase; NMT—nonspecific methyltransferase; AAD—aromatic acid decarboxylase; PNMT—phenylethanolamine-N-methyltransferase; CFE—catecholamine-forming enzyme.

Figure 1. The Biosynthesis of the Catecholamines (13)

role of epinine in the CNS except that it may be a precursor of adrenaline. As DA, noradrenaline, and adrenaline do not cross the blood brain barrier it is unlikely that epinine does. Exogenously administered epinine acts as an effective sympathomimetic in the periphery but it has little if any central action (33).

Transmethylation Processes and Psychosis

Attempts have been made to correlate these biochemical findings with clinical situations. Much of this has been reviewed by Kety (15) and Boulton (16). Narasimhachari, Plaut, and Himwich (17) looked in human serum for an enzyme capable of N-methylating serotonin. In a small series, they found no activity in the serum of their normal controls, but of seven samples from acute schizophrenics, six were positive for either bufotenin or 5-methoxy-N-N-dimethyltryptamine. Wyatt et al (18) have found an enzyme capable of forming DMT in red blood cells, plasma and platelets. The enzyme activity in the red blood cells and plasma was similar in psychiatric patients and normal subjects but the enzyme activity in the platelets was higher in psychotic subjects than in nonpsychotic subjects. N-methyl transferase activity of the platelets could be increased by dialysis, implying the presence of an inhibitor.

Hoffer (19) treated schizophrenic patients with the methyl acceptors niacin and nicotinamide in an attempt to inhibit the abnormal methylation that might be occurring. Their partial success led to a controversial chemotherapy for schizophrenia (20). Pollin, Cardon, and Kety (21) tested the theory one step further by studying the effects of feeding large doses of methionine and tryptophan to schizophrenics. The amino acids caused behavioral symptoms, some of which seemed to be exacerbations of the psychoses. The findings were confirmed by several independent groups, including one using betaine as a methyl donor (22). Feeding methionine to nonpsychotic patients also caused behavioral changes (23). It is not known, however, whether the resulting symptoms are truly an intensification of the pre-existing psychosis, or a toxic psychosis, or if an abnormal methylation is even involved.

Friedhoff and Winkle (24) reported that they had found 3,4-dimethoxyphenylethylamine (DMPEA), which became known as the pink spot, in the urine of schizophrenics. This report began one of the most controversial issues in the biochemistry of schizophrenia. The pink spot is critically reviewed by Siegel (25), who found it related to chlorpromazine ingestion. Stabenau (26) observed that DMPEA can be produced in the urine of both normal subjects and schizophrenics provided they drink tea. Narasimhachari et al (27), using a more selective and

specific test for DMPEA, were unable to find it in the urine of their psychiatric patients and controls whether or not they were tea drinkers.

In addition to these major issues there are many other studies attempting to relate methylation processes to schizophrenia. Abnormal methionine metabolism, for example, has been reported in schizophrenic patients (28), and schizophrenia has been found in a number of families with homocystinuria (29).

5MTHF as a Methyl Donor

Laduron discovered the 5MTHF dependent methylating enzyme while looking in rat brain for an extra-granular N-methyl transferase that would obviate the seemingly uneconomical translocations of noradrenaline and adrenaline from the granules to the extra-granular space and back again (30,31). Unlike the extra-granular dopamine-N-methyl transferase he found (30) in the adrenal medulla, which uses SAM as a methyl donor, the enzyme in rat brain was able to use only 5MTHF.

5MTHF has since been observed (34) to participate with greater activity than SAM in the N- and O- methylation of phenylethylamines and indoleamines by extracts of mammalian and avian tissues (see Table II). Among the *in vitro* products formed by the 5MTHF dependent enzyme are the psychotomimetic agents bufotenin and 5-methoxy-N-N-dimethyltryptamine.

Table I. Species and tissue distribution of methyltransferase activity. Tissues were homogenized in ten volumes of 5 μM sodium phosphate buffer (pH 7.9). After dialysis, the solutions were centrifuged at 100,000*g* and the supernatant was assayed for enzyme activity. Serotonin (5 mM) (S) or tyramine (5 mM) (T) were substrates and S-adenosylmethionine (1 μM) or 5-methyltetrahydrofolic acid (1 μM) were methyl donors. Data are presented as the mean of three experiments whose results varied less than 20 percent. Enzyme activity is expressed as picomoles of methyl group added to the substrate per milligram of protein in 1 hour.

Tissue	AMe			MTHF			Ratio of enzyme activity with AMe to activity with MTHF	
	S	T	S/T	S	T	S/T	S	T
Rabbit lung	32.0	24.0	1.33	8.0	4.0	2.0	4.0	6.0
Rabbit brain	0	0.05	0	2.6	1.4	1.9	0	0.04
Rabbit liver	0	0.2	0	2.1	1.2	1.7	0	0.03
Rat brain	0	0.4	0	3.0	1.3	2.3	0	0.31
Rat liver	0	1.2	0	4.3	1.4	3.1	0	0.90
Rat lung	0	0.35	0	4.0	1.3	3.1	0	0.27
Rat heart	0	0.4	0	8.0	3.0	2.7	0	0.14
Chick brain	0	1.0	0	6.0	4.0	1.5	0	0.25
Chick heart	0	2.0	0	26.0	11.0	2.3	0	0.18

74

Table II. Comparison of SAM and 5MTHF dependent methyl transferase enzyme activities (34)

These findings introduce the question of the role of folate coenzymes in neural metabolism and psychiatric illness.

Folic Acid Metabolism

While most dietary folates are pteroylpolyglutamates (Figure 2), the predominant form of folate in the serum is 5MTHF, for during absorption hydrolysis, reduction, and methylation occur (35). In addition to 5MTHF there are five other folate coenzymes which are one-carbon derivatives at either the N^5 or N^{10} position or both as shown in Table III (36). Little is known about the utilization of folate coenzymes in neural tissue, but it is known that the concentration of 5MTHF in the cerebrospinal fluid (CSF) is 3-4 times the concentration of 5MTHF in the serum (37), that there is a selective conservation of 5MTHF in the CNS (38) as opposed to a selective exclusion of vitamin B_{12} (36), that 5MTHF is taken up preferentially in CSF while other folate congeners are converted to 5MTHF before uptake (39), and that there is an association of disturbances in folic acid levels and neuropsychiatric disorders (43-66).

Folate coenzymes are intricately involved with vitamin B_{12} metabolism and take part in methyl group transfer reactions including:

1. the synthesis of purines (10-formyl THF and 5,10-methenyl THF)
2. the synthesis of the pyrimidine thymidylate (5,10-methylene THF)
3. the interconversion of serine and glycine (THF, 5,10-methylene THF)

4. the catabolism of histidine to glutamic acid (THF)

5. the methylation of homocysteine to methionine (5MTHF) (36)

Figure 3 summarizes these reactions. In addition, reduced pteridine proups are required as cofactors in the metabolism of phenylalanine to tyrosine, and tyrosine to dopa, the first steps in the biosynthesis of dopamine, noradrenaline, and adrenaline (13).

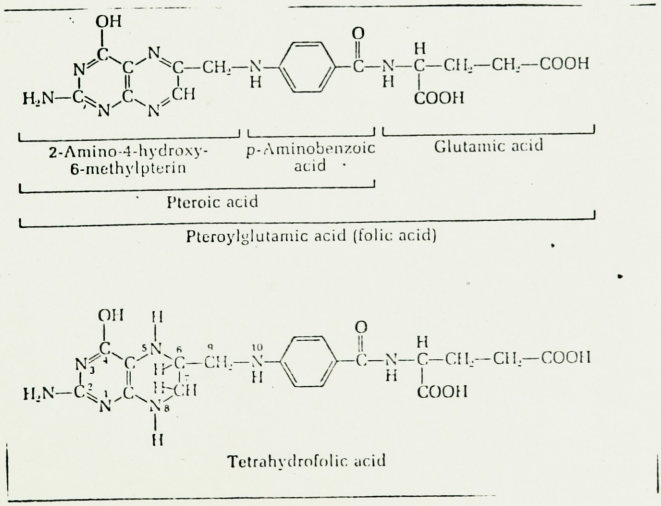
The folate coenzymes are all interrelated as shown in Figure 3 (42). THF is produced as the coenzymes donate carbon groups in the various reactions and by the reduction of dihydrofolate by the enzyme dihydrofolate reductase, which is strongly inhibited by the chemotherapeutic drug methotrexate (40). The major pathways for the methylation of THF involve the reactions 3 and 4 above and direct formylation using THF formylase.

The major reaction in human metabolism in which 5MTHF is known to act as a coenzyme is the methylation of homocysteine to methionine (24). There is one theory, in fact, that postulates that 5MTHF can be converted to other folate congeners only via this reaction (see methyl trap hypothesis below). This reaction is also the only known reaction in human metabolism which involves both folate and vitamin B_{12} and thus is important in the etiology of megaloblastic anemias and neuropsychiatric disorders associated with B_{12} and folate deficiencies.

Table III. Derivatives of Tetrahydrofolic acid (36)

Derivative	R	Oxidation State
N^5 formyl THF	-CHO	formate
N^{10}formyl THF	-CHO	formate
N^5formimino THF	-CH=NH	formate
$N^{5,10}$methenyl THF	$\diagdown\!\!\!\!\diagup$CH	formate
$N^{5,10}$methylene THF	$\rangle CH_2$	formaldehyde
N^5 methyl THF	$-CH_3$	methanol

Figure 2. Structure of Folic Acid and Tetrahydrofolic acid



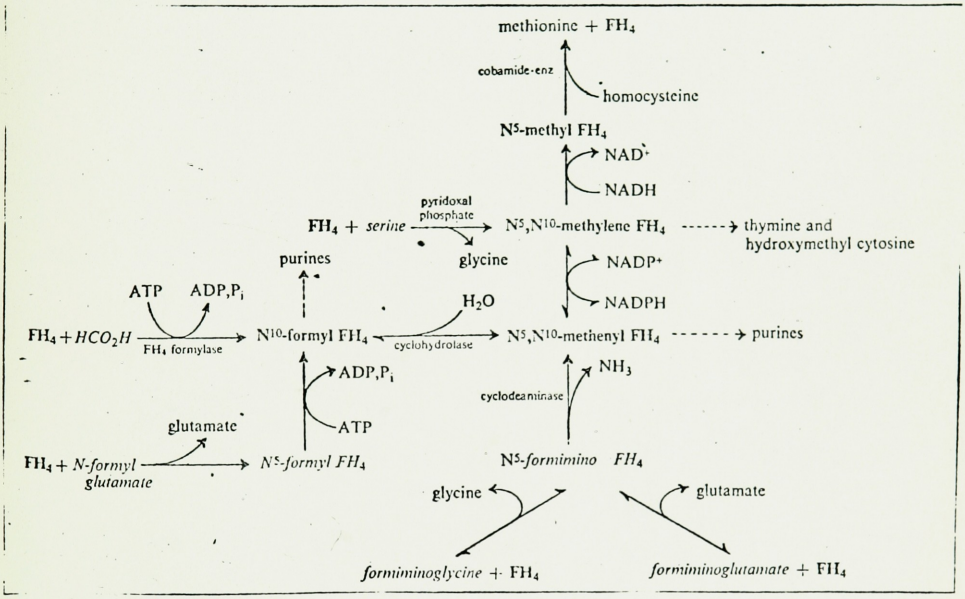


Figure 3. Metabolism of Tetrahydrofolic Acid (42)

Homocysteine is methylated to methionine via two possible systems. One, found in bacteria and not known in humans, is independent of vitamin B_{12}. The other, found in bacteria and humans, is dependent on the presence of vitamin B_{12} as shown in Figure 4 (40,41). The cobalamin dependent reaction was the first and most extensively studied reaction. The cofactors ATP, Mg^{2+}, and $FADH_2$ or its equivalent are necessary for the reaction. The reaction

5MTHF + homocysteine $\longrightarrow$ THF + methionine

is catalyzed by homocysteine methyltransferase. The apoenzyme is converted to the holoenzyme when it combines with the cobalamin prosthetic group that receives the methyl group from 5MTHF and gives it to homocysteine. The methyl transfer is stimulated by SAM (41).

The methyl trap hypothesis is the most accepted theory for the etiology of megaloblastic anemia (35). The theory holds that the lack of vitamin B_{12} prevents 5MTHF from participating in homocysteine methylation. Folate accumulates as 5MTHF, reducing the amounts of folate coenzymes needed in other reactions. When these fall below a threshold, nucleic acid formation fails. This theory is not accepted by all, but at the present there is no better explanation of the role of folate and vitamin B_{12} in megaloblastic anemias (40).

The non-cobalamin pathway in bacteria and lower animals uses only 5MTHFdiglutamate and Mg^{2+}, and is not known to occur in humans. It is interesting to note the close relationship

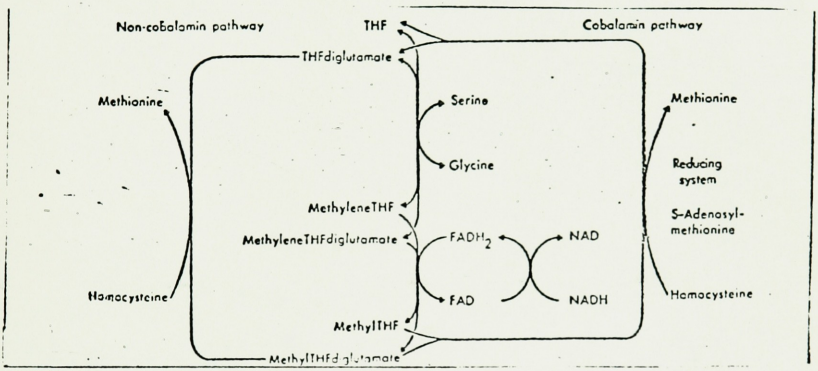


Figure 4. Cobalamin and Non-cobalamin dependent pathways for Methionine Biosynthesis (40)

between 5MTHF and methionine, particularly in light of the methionine feeding experiments mentioned before. It has been found that methionine feeding in cobalamin-deficient animals and humans greatly decreases fromiminoglutamate excretion in addition to decreasing both the amount of 5MTHF relative to other folates in liver and the level of total folates in liver (40).

Folates and Neuropsychiatric Disorders

Laduron (3) suggests that since 5MTHF has been found to take part in the metabolism of biogenic amines, it is possible that an excess of folate coenzymes could give rise to an increased or unusual formation of N-methylated amines. Folic acid deficiencies and toxicities have been implicated in neuropsychiatric disorders, mainly in patients receiving the anti-convulsant drugs diphenylhydantoin, phenobarbital, and primidone. Reynolds (43,44) and Rothfeld (45) have reviewed the literature.

Three studies (46-48) found significantly lower serum folate levels in epileptic patients with mental illness than in epileptic patients with normal mental states. In two of these studies (47,48) psychotic (dementia, schizophrenia-like, depression) patients had lower serum folate levels than non-psychotic epileptics. Other studies have shown that reduced

serum folate levels are accompanied by corresponding changes in the red cell folate levels (49) and in the CSF (50, 51). Horvitz et al (52) take exception, finding low levels in all his epileptic patients with no significant difference between epileptic patients with and without neuropathy.

Treatment with folic acid has yielded conflicting results. Reynolds (53) treated 26 chronic epileptic patients with folic acid deficiency due to anticonvulsant drugs and found that mental state improved in 22 with increased drive, speed of cerebration, alertness, self-confidence, independence, and sociability. Neubauer (54) found improvement in mental state in folate deficient epileptic children 5-8 weeks after beginning treatment with folic acid and vitamin B_{12}.

On the other hand, Spaans (55) in a controlled double blind study found that after three months of folic acid therapy, serum folate levels had risen but CSF folate levels showed no rise. Mattson et al (56) treated epileptic patients in a double blind study with folic acid for six months and found no change in CSF folate levels, no change in seizure activity, and no improvement in mental activity. Since it had been shown that diphenylhydantoin did not alter uptake of 5MTHF into the CSF (38), it was postulated by Mattson et al that the anticonvulsants interfered with conversion of folic acid to 5MTHF. When a small group was treated with folinic acid (5 formylTHF) CSF levels rose rapidly.

Folate deficiencies have been implicated in non-epileptic neurological and psychiatric patients. Reynolds, Rothfeld, and Pincus (57) studied the neurological status of acute general medical patients with and without serum folate deficiency, controlling for degree of anemia and presence of alcoholism. The folate deficient group had a significantly larger number of organic brain syndromes and pyramidal tract changes. Pincus, Reynolds, and Glaser (58) reported a woman whose symptoms of subacute combined system degeneration: megaloblastic anemia, dementia, impairment of posterior column function, absent reflexes, and bilateral Babinski signs, all disappeared with folic acid therapy. Strachan and Henderson (59) reported two patients with megaloblastic anemia and advanced dementia; both markedly improved with folic acid therapy. In a retrospective study, Carney (60) found that psychiatric patients with organic psychosis, endogenous depression, and schizophrenia who had been given folic acid were discharged more quickly and in a better clinical state that those who had not received folic acid. It has been found that inborn errors in folate metabolism can cause mental retardation (61).

In addition to these studies on the effect of folic acid deficiency, Hunter et al (62) report the toxicity of folic acid. 15 mg/day of folic acid was given to a group of 14 healthy volunteers. The study had to be stopped after one month because most subjects had GI or nervous symptoms or both. This study was not controlled, however, and the

symptoms listed were varied and questionable.

Hunter (63) suggests an explanation for his findings. Noting that pteridine cofactors are required in brain amine metabolism, he postulates that excess folate may inhibit the enzyme dihydrofolate reductase and thereby decrease the amount of biologically active THF. As supporting evidence he notes that he found CSF homovanillic acid levels were lowered by folic acid ingestion. This does not, however, say anything about THF levels or enzyme inhibition, nor is any mechanism for HVA reduction proposed.

Hellstrom (64) followed this report with a controlled double blind study. Psychic reactions were measured with a questionnaire. No differences were observed between controls and subjects. Hellstrom notes, however, that the serum folate levels in her patients were much lower than in Hunter's study.

Reynolds (65) has reviewed the evidence that increases in folate levels may cause an increase in seizure disorders. In one study he found an exacerbation of seizures in 50% of his patients, the longer the follow up the more the deterioration in seizure control. Mattson et al (56) found six months therapy with folic acid had no effect on seizure frequency or severity. In support of a theory that focal epileptic discharges may result from the accumulation of folate at the site of the focus as a result of local damage to the blood brain barrier, Mayersdorf et al (66) have found folate

concentrations in the region of chronic cobalt epileptic foci to be several times higher than in other areas of cortex.

The evidence implies that folic acid plays an important and complex role in neural functioning. Folate levels in serum, RBC, and CSF are significantly low in various forms of mental illness, especially psychoses, but not all epileptic patients with mental illness have low folate levels and not all epileptic patients with low folate levels have mental illness. CSF levels correspond with serum levels except that treatment with folates may not affect CSF folate levels. It is difficult then, to account for the improvement in mental status seen in the various studies.

Dopamine and Serotonin Pathways in the Brain

The new methyl transferase and its interactions with 5MTHF may be important in elucidating the role of folic acid in the nervous system. The study of the regional distribution of the enzyme in the brain with special attention to its relationship to dopamine and serotonin pathways seemed especially interesting for three reasons: the role of epinine in the biosynthesis of catecholamines, the evidence that dopaminergic neurons in the corpus striatum may be involved in schizophrenia (67), and the ability of the enzyme to form psychotomimetic derivatives of serotonin.

The monoamine pathways in the central nervous system have been the subject of numerous studies, particularly by Dahlström, Fuxe, and Ungerstedt. (68-74) who used fluorescence histochemical techniques. There are three major DA pathways as mapped by Ungerstedt (74). The nigro-striatal path arises in the zona compacta of the substantia nigra, ascends in the lateral hypothalamus, enters the crus cerebri in the mid-hypothalamus, intermingles with the myelinated bundles in the capsa interna, fans out in the globus pallidus, and enters the caudate. Approximately 12% of the nerve terminals in the caudate are dopaminergic (75). The meso-limbic DA system ascends with the axons of the nigro-striatal DA system, but takes a more medial route to the nucleus accumbens, nucleus interstitialis striae terminalis, and the olfactory tubercle. The tubero-infundibular DA system arises in the arcuate nucleus in the hypothalamus and spreadsto the median eminence, hypothalamus, and perhaps higher brain areas. The 5HT cell bodies arise in the dorsal raphe nucleus in the midbrain and ascend to the forebrain innervating particularly the hippocampus and cortex. A small ($\angle$1%) amount of the cells in the forebrain contain serotonin (76).

These findings have been confirmed in many laboratories (77-82). Heller and Moore (77) showed that there is a large decrease in brain serotonin after destruction of the raphe nuclei. Other laboratories have found decreases in DA when the medial forebrain bundle or the substantia nigra is lesioned (78,79,81). The effects of electrical stimulation

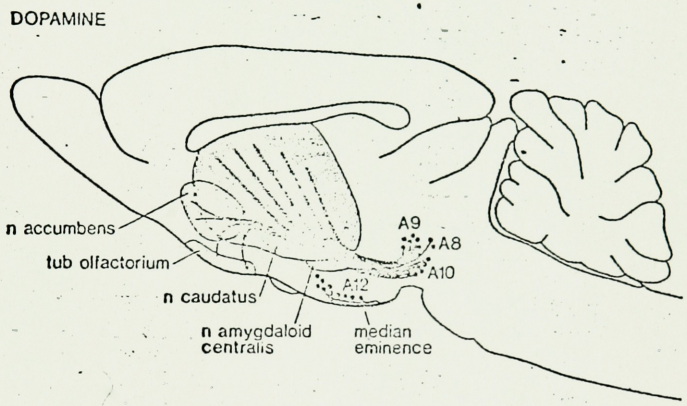


Figure 5. Sagittal projection of Dopamine pathways in brain. (74)

of the substantia nigra on the caudate have been correlated with the effects of iontophoretic DA on caudate neuron firing rates (80).

The monoamine pathways correlate with the regional distribution of monoamines in the brain (83). It is significant that DA levels are highest in the corpus striatum (see Table IV).

Table IV. Regional Distribution of Monoamines in Brain adapted from Valzelli and Garattini (83)

Brain area	5HT ug/g	NA ug/g	DA ug/g
Whole brain	0.37	0.28	0.49
Hemispheres	0.25	0.17	0.50
Corpus striatum	0.30	0.71	5.25
Cerebellum	n.m.	0.14	n.m.
Frontal Cortex	0.10	0.19	0.09

n.m.=not measurable

Methods

Preparation of C^{14}-5MTHF

50 µCi of Ba:C^{14}-5MTHF (Specific Activity 57 µCi/µmol, Amersham) is taken up in 1.5 ml 0.1 M sodium bicarbonate. 2.5 ml sodium ascorbate (5 mg/ml, pH adjusted to 6 with NaOH) is added and followed by 1.0 ml disodium phosphate. The solution is centrifuged at 1000 X g and the supernatant is divided into vials.

Assay of N-methyl transferase activity

The brains of male rats (Charles River, 200-300 g) were homogenized in four volumes of 0.1 M Tris buffer at pH 7.9. The homogenate was centrifuged at 16,000 x g for 15 min. The N-methyl transferase activity of the supernatant preparation was then assayed in an incubation mixture containing 0.1 M Tris buffer at pH 7.9; 20 µmol DA substrate; 0.5µCi C^{14}-5MTHF; 5 µmol EDTA; 8 µmol dithiothreotol; and a sample of supernatant in a final total volume of 1 ml. Boiled supernatant was used as a blank. The mixture was incubated for 1 hour at 37° C. Each reaction was stopped with the addition of 3 ml of 10% trichloroacetic acid; and the mixture was centrifuged at 36,000 X g for 15 min. 50 µg carrier epinine, 0.4 ml 10% EDTA, and 0.2 ml 1 M Tris buffer at pH

8.4 were added to each sample; the pH was adjusted to 8.4 with NaOH; and the samples were poured over columns of aluminum oxide (85). After washings with 30 ml H_2O, the amines were eluted with 2 ml 1 N HCl followed by 2 ml H_2O. An aliquot of the eluate was counted with 10 ml of scintillation solution in a Packard liquid scintillation counter.

Chromatography

The remainder of the eluate from three experiments was poured over alumina columns a second time, eluted with 0.2N PCA, and subjected to ion exchange column chromatography (86) for identification of the radioactive catecholamines.

Lowry Proteins

Protein measurements as described by Lowry et al (87) were performed on whole rat brain and caudate, and human caudate samples.

Enzyme Activity

DA, epinine, folate, and supernatant concentrations were varied to study the effects on the reaction. Time studies were done from 15 to 150 min. The regional distribution of the enzyme activity was studied by dissecting and separately homogenizing and assaying supernatant fractions of the cortex,

the hippocampus, cerebellum, and caudate. In one experiment, a tissue slice containing the raphe nucleus was assayed. Enzyme activity was measured in fresh tissue and tissue that had been frozen from one hour up to three months.

Human Caudate Enzyme Activity

Three pieces of humand caudate, about .2 g each, were obtained from a 56 year old woman who had died of metastatic ovarian carcinoma. The samples were frozen (-20° C) for 48 hours, homogenized and assayed as described above. A fourth piece was assayed after being frozen for 2 months.

Lesioning of Medial Forebrain Bundle and Raphe Nucleus

The rats were lesioned as described by Walters (81). They were anesthetized with halothane and mounted in a stereotaxic device. The skull was exposed, and a 3 mm burr hole was made on the left side at 1300 μ lateral and 2790 μ anterior according to Konig and Klippel (82). A metal electrode made from a #1 insect pin, coated three times with and then scraped ½ mm clear of insulation at the point, was lowered 8.3 mm from the skull surface. A 20 sec electrothermic lesion was mad with a Grass LM4 Lesion

Maker set at 15-20 mAmp. The wound was clipped shut and the animals were allowed to recover from the anesthetic. Nine days after the lesion the rats were decapitated and the caudates were dissected out with care to keep the left (lesioned side) and right (unlesioned) side separate. Each caudate was assayed separately for enzyme activity. The assay procedure was the same as above, except that only 0.3 - 0.5 ml supernatant was available for the assay due to the small amount of tissue.

The raphe lesion technique was similar except that the coordinates used were anterior 340 μ, lateral 0 μ, and horizontal 6.5 mm and 7.5mm (84). As the raphe lesion affects both caudates, unlesioned rat brains were used as controls. In this experiment, the caudates were homogenized in 10 volumes of buffer.

Histology was performed on the lesioned brains to confirm the location of the lesion. The tissue was placed in a 5% gluteraldehyde solution for 48 hours. Sections were made at 50 μ intervals with a microtome freezing stage, and mounted on glass slides smeared with albumin. The sections were stained with crystal violet solution: slides were dipped successively into staining dishes containing 95% ethanol, distilled H_2O, 0.1% crystal violet acetate (Fisher) for 5 min, distilled H_2O, 95% ethanol with a few drops of acetic acid, absolute ethanol, 50:50 solution of absolute ethanol:xylene, absolute ethanol, and xylene. The slides were examined to determine location and size of the lesion. If the extent and location of the lesion were not correct the biochemical data from the

improperly lesioned brain was not used.

Ammonium Sulfate Purification of N-methylating enzyme

In one experiment, partial purification as described by Laduron (3) was attempted. Three rat brains were homogenized in 30 ml H_2O, and centrifuged at 16,000 X g for 10 min. The supernatant was centrifuged at 130,000 X g for 90 min to obtain the supernatant which was partially purified with a precipitation at a 25% ammonium sulphate saturation. The pellet was discarded and the supernatant added to a solution at 60% ammonium sulphate saturation. The second pellet was taken up in 0.02 M phosphate buffer at pH 6.8, dialized overnight against the same buffer, and then centrifuged again at 20,000 X g for 20 min before being frozen for storage before assay.

Results

Whole Brain Studies

The 5MTHF dependent dopamine-N-methyl transferase was found to be active in the rat brain. The reaction was followed by measuring the change in product as measured by radioactivity (DPM) in the eluate from the aluminum oxide columns. Figure 6 shows the reaction product when 0.8 ml supernatant is incubated from 15 to 150 minutes with the reaction mixture described above. The product increased

linearly with time up to 90 min when it quickly leveled off.

Figure 7 shows the results when time was held constant at one hour and the amount of supernatant containing enzyme was varied from 0.2 ml to 0.8 ml. The amount of product increased linearly as more enzyme was added to the reaction mixture.

In the experiment shown in Figure 8, the amount of DA substrate was varied from 175 μg to 7 mg. The reaction rate increased rapidly as the substrate concentration increased to about 2 mg when the enzyme must begin to become saturated. This curve is typical of an enzyme-catalyzed reaction which follows Michaelis-Menten kinetics. As the radioactivity decreased at high substrate levels, it is possible that the reaction direction was reversed. Epinine was also tried as a substrate and found to accept a methyl group, but chromatography was not performed to determine the product nor was the reaction investigated further.

In Figure 9, the DA was held constant while the 5MTHF was varied from 0.1 to 1 uCi (0.018 umol to 0.18 umol). The reaction product increased linearly with the coenzyme until a saturation point was reached at about 0.5 uCi.

Ion exchange chromatography was performed to identify the reaction products. H^3-DA and standards of epinine and noradrenaline were used for comparison, as Laduron (3) reported that the epinine peak in column chromatography

overlaps the DA peak. In these experiments, as shown in Figure 10, the C^{14} came off just after the H^3-DA peak, findings consistent with standard controls. No other peaks were found in the experimental samples.

Regional Distribution

In five separate experiments, the enzyme activity determined as DPM/mg tissue wet weight was measured for the cortex, hippocampus, cerebellum, and caudate. The number of trials varied from 3 to 8. The cortex had the lowest amount of enzyme activity/mg tissue, and the cortical enzyme activity was selected as a reference value and set at 100%. As shown in Table V, the caudate consistently had the most enzyme activity, about twice as much as in the cortex. In a single experiment, tissue slices containing the raphe nucleus were homogenized,assayed, and found to have less activity than the cortex.

Enzyme Activity

The enzyme activity in the caudate lobe, as shown in Table VI, was found to be 0.112 mumol pdt. formed/ mg protein. This is about 3 times the activity found by Laduron in whole rat brain. The enzyme activity in the human caudate tissue was found to be 0.115 mumol pdt. formed/mg protein. It was found that the enzyme activity in both human and rat caudates was changed very little when the tissue was frozen up to two months before being assayed.

The ammonium sulphate precipitation increased the enzyme concentration, but it was decided that this procedure was unnecessary for this study. It is important to note, however, that the enzyme was present in the soluble fraction after the ultracentrifugation.

Lesion Studies

When the medial forebrain bundle was lesioned, no significant changes in the enzyme activity in the caudate was found. The enzyme activity in the lesioned and unlesioned sides is presented in Table VII. In addition, Table VIII shows the data from the caudates when the raphe nucleus was lesioned. Again, there was no significant change in enzyme activity.

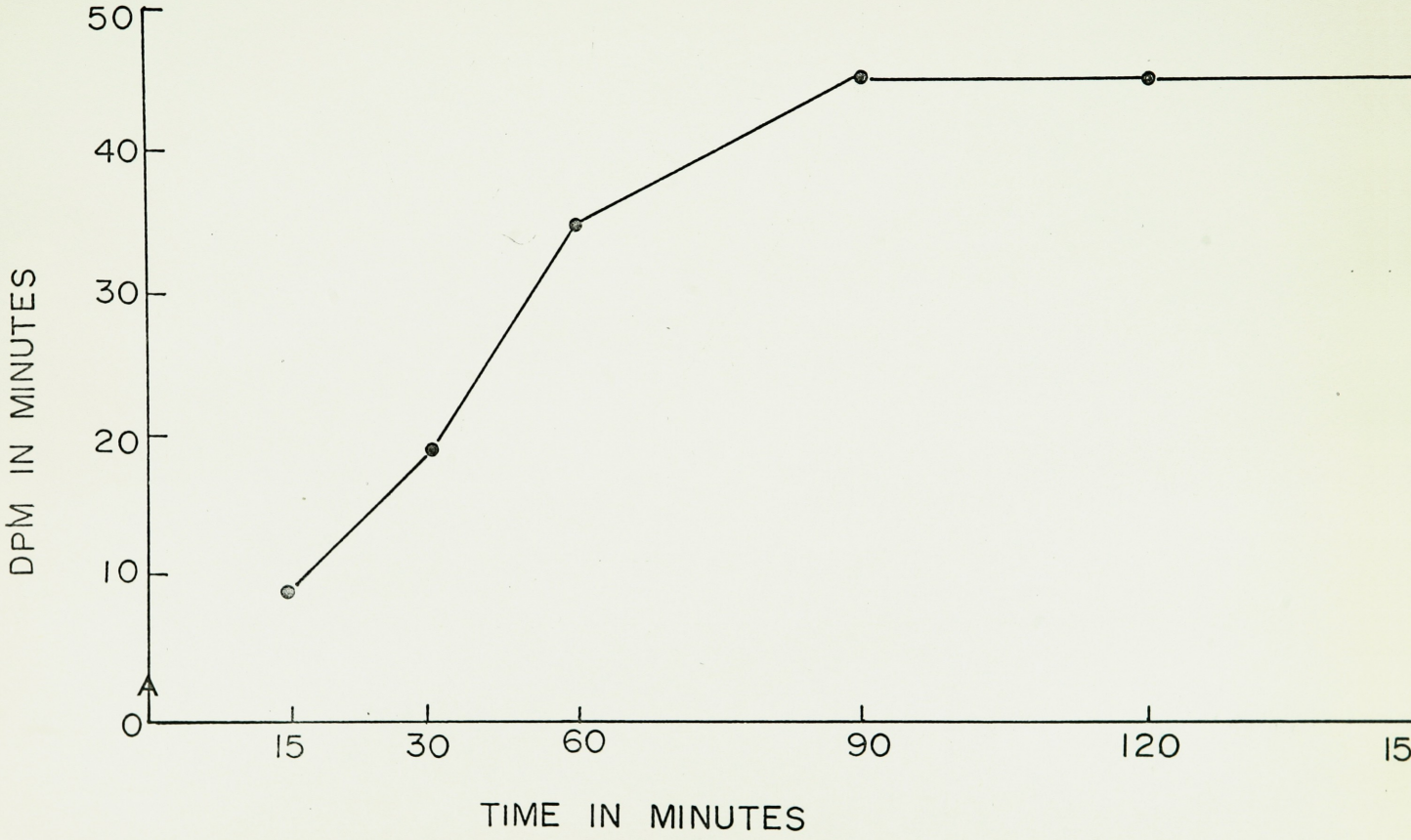


Figure 6. Effect of Time on Reaction Product (radioactivity)

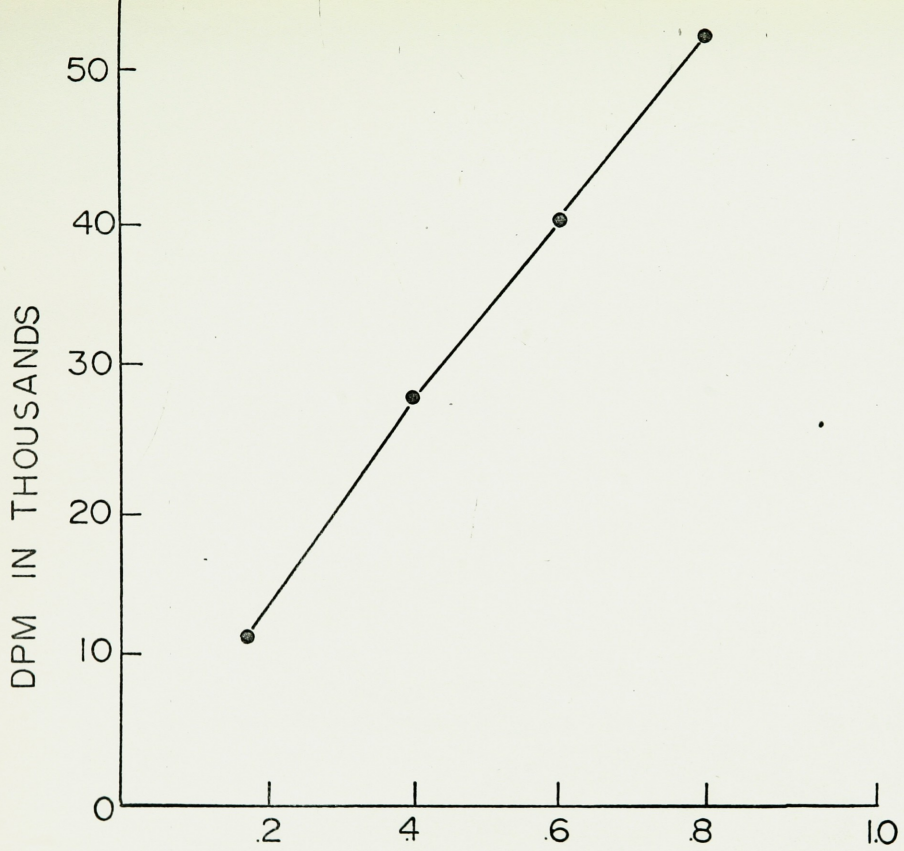


Figure 7. Effect of Enzyme Concentration (ml supernatant) on Reaction Product

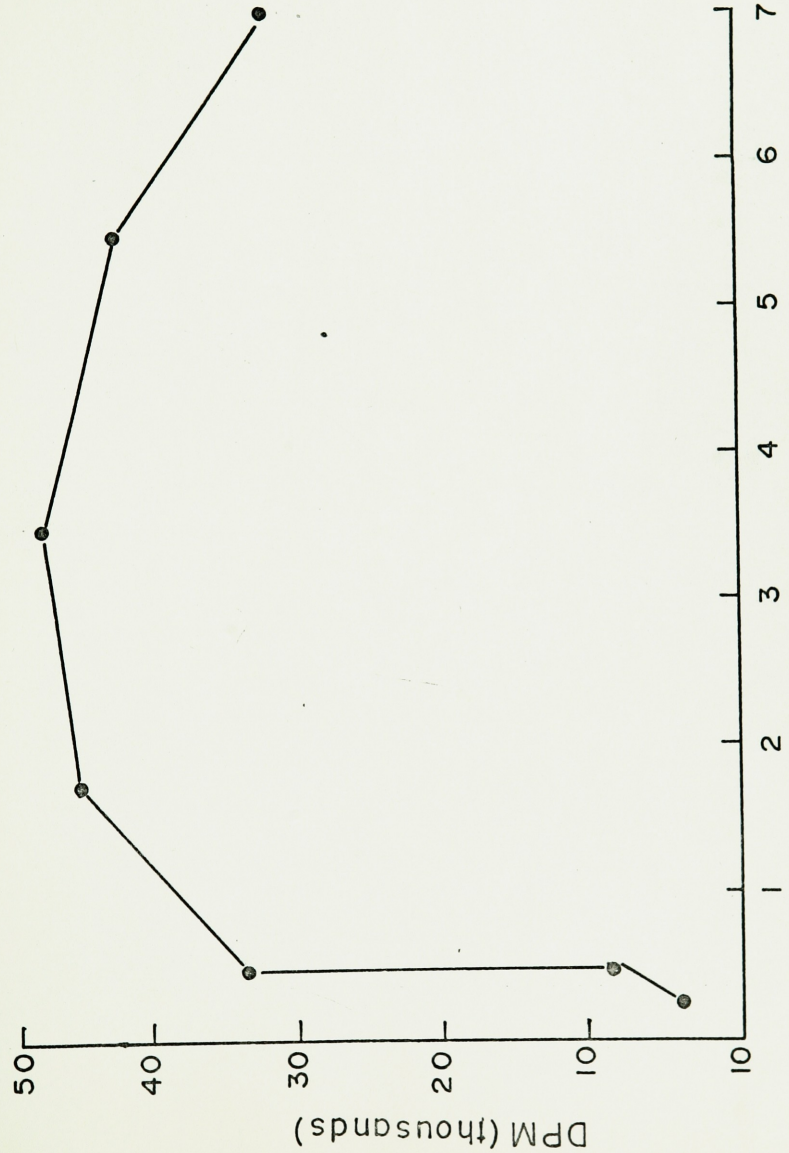


Figure 8. Effect of Substrate Concentration on Reaction Product

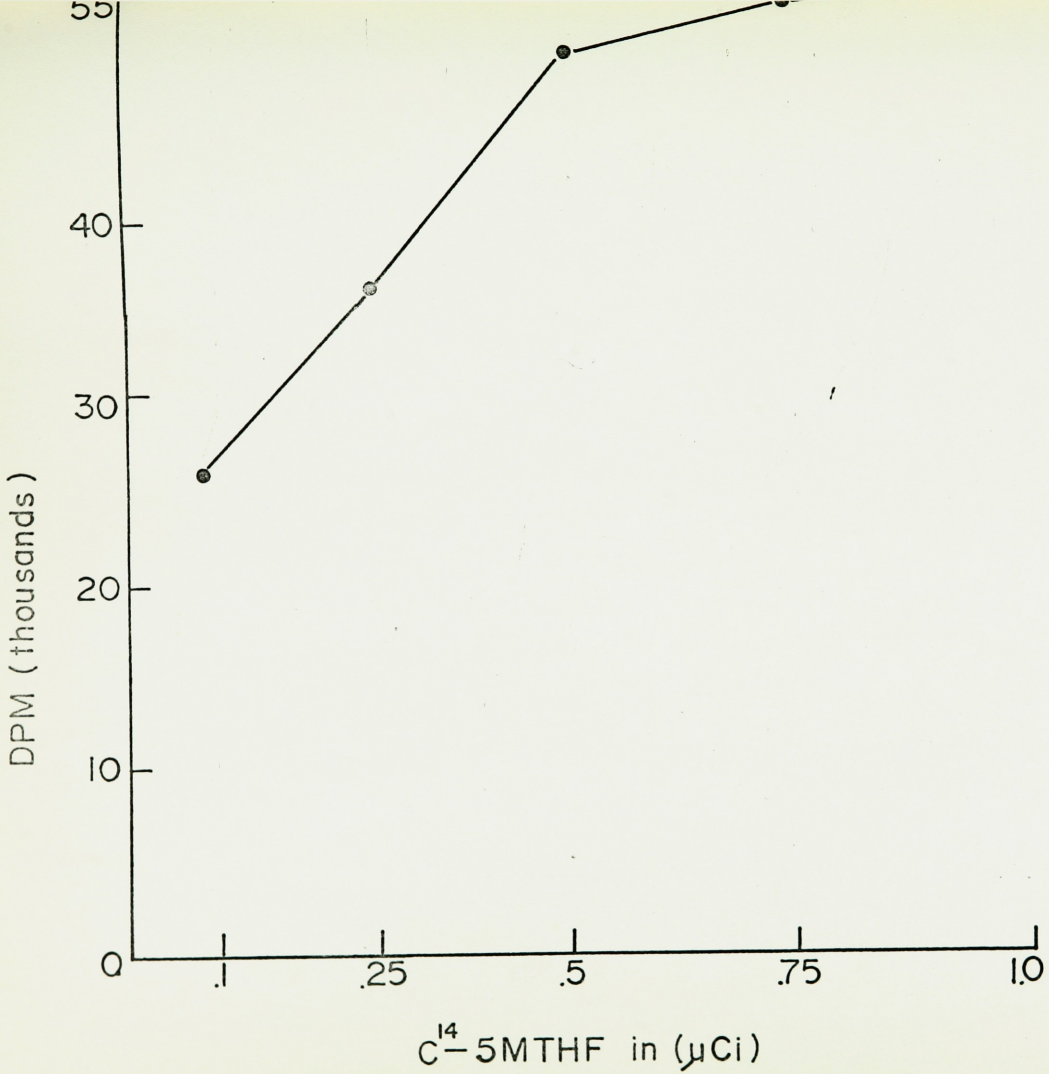


Figure 9. Effect of Coenzyme Concentration on Reaction Product

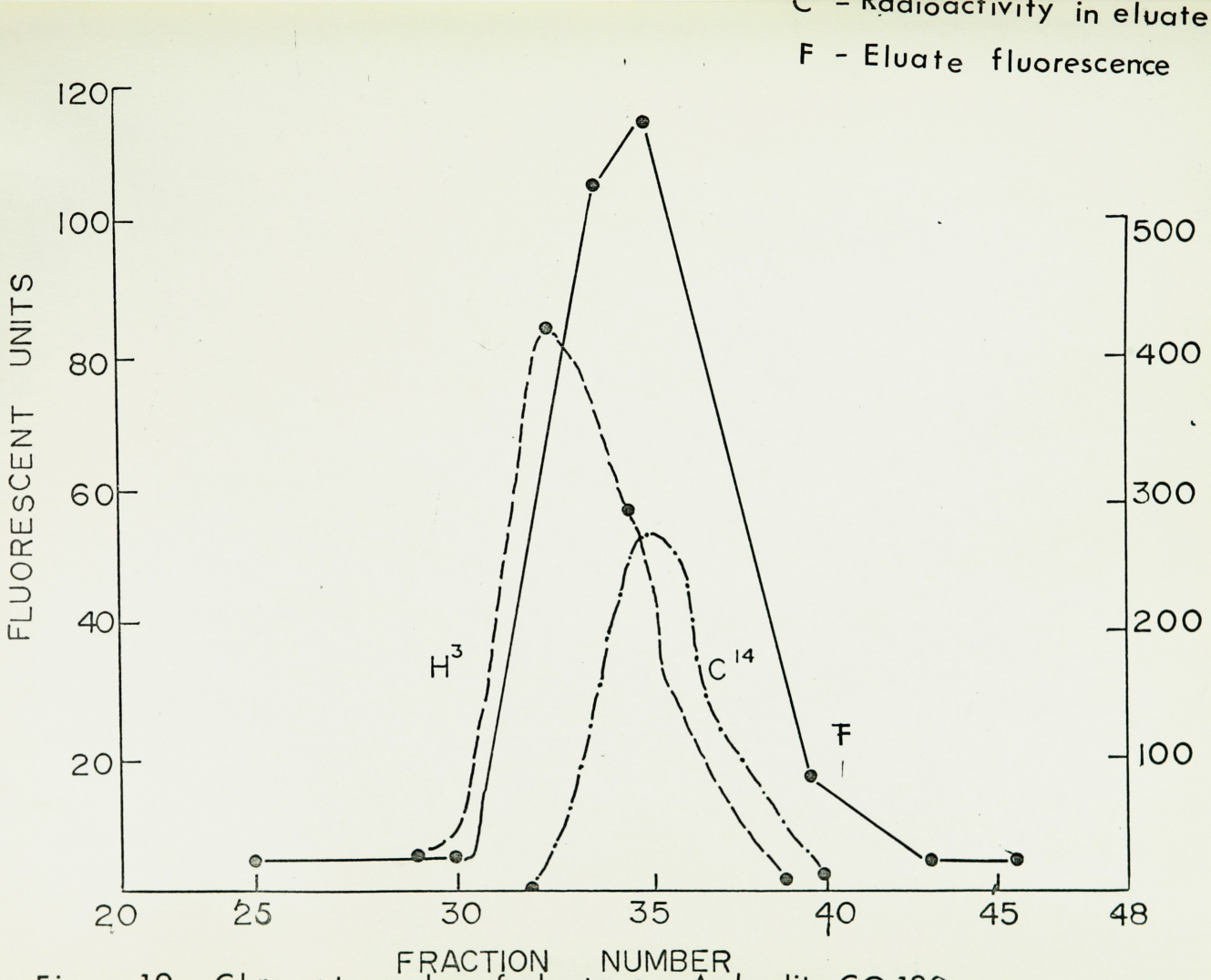


Figure 10. Chromatography of eluate on Amberlite CG-120

Table V. % 5MTHF Dependent-N-Methyl Transferase Activity in Brain Areas compared to cortex (100% ± 15%)

Brain Region	Mean %	SE	Significance
Cortex	100	15	
Caudate	202	18	$P < .005$
Cerebellum	169	13	$P < .02$
Hippocampus	118	10	n.s.
Raphe	92	--	(one experiment)

n.s.-not significant

Table VI. Enzyme Activity expressed as mumol of product formed in one hour/mg protein

Tissue	mumol/mg protein
rat caudate	0.112
human caudate	0.115
per Laduron (3):	
rat brain	0.036
rat adrenal medulla	0.087

Table VII. Caudate enzyme activity after medial forebrain bundle lesions

	DPM/mg wet weight	
Rat	Left (lesioned side)	Right (unlesioned side)
1	209	197
2	78	111
3	301	262
4	249	259
5	285	320
6	253	169
7	81	73
8	79	87
Mean	192 ± 35	151 ± 32 (n.s.)

Table VIII. Caudate enzyme activity after raphe lesions

DPM/ mg protein

Lesioned Rats	Unlesioned Rats
4.65	10.37
10.15	18.98
13.38	18.99
16.74	10.54
12.57	11.58
8.20	
9.48	
10.23	
Mean 10.67 ± 1.28	14.09 ± 2.0 (n.s.)

Discussion

In initial experiments we observed that it was possible to assay the 5MTHF dependent DA-N-methyl transferase without the prior ammonium sulphate precipitation procedure used by Laduron (3). This greatly facilitated the study of the regional distribution of the enzyme.

The major problem presented by the enzyme assay described by Laduron was the development of a black discoloration during the incubation. Laduron (30) mentions this discoloration as most likely caused by the oxidation of the DA substrate, and notes that EDTA and sodium metabisulfite prevent the oxidation from taking place. In the present studies, high concentrations of EDTA and sodium metabisulfite did not protect the reaction mixture from turning black. The problem was solved only with the addition of dithiothreotol, an effective reducing agent, which prevents the formation of the black discoloration and allows for greater enzyme activity.

The 5MTHF dependent DA-N-methyl transferase has been shown to be most concentrated in the caudate lobe as compared with the cortex, hippocampus, cerebellum, and raphe. Korevaar et al (88) have found high levels of 5MTHF in the caudate. The finding of enzyme and associated coenzyme to be localized in high concentration to the same areaof the brain

argues for the significanceof their reactions--the alternate pathway for the biosynthesis of adrenaline via epinine and other N- and O-methylation reations catalized by the folate dependent enzyme.

The high levels in the corpus striatum are also interesting because the striatum has been implicated in the pathophysiology of schizophrenia (67).

What then is the physiologic and, if any, behavioral role of the folate dependent methyl transferase? The first step to answering this question is to try to find if the enzyme is localized to a particular kind of cell. There was, however, no clear cut localization to either DA or 5HT containing neurons. When the medial forebrain bundle was lesioned (producing degeneration of DA neurons distal to the site of the lesion) there was no significant change in the enzyme activity in the caudate. If DA were the main substrate and if epinine were made in the cells, one would expect a decrease in the enzyme activity when the DA axons degenerated.

Banerjee and Snyder (34) have reported that the folate dependent enzyme affinity is greater for serotonin than other biogenic amines, and have suggested that the physiologic role for this enzyme may involve the N- or O- methylation of serotonin. When the raphe was lesioned, however, there was no decrease

in the enzyme activity in the caudate as measured by the N-methylation of DA.

This data implies that the enzyme is not found exclusively in the DA or 5HT axons in the caudate. DA and 5HT terminals account for only 13% of the neuronal terminals found in the caudate. The enzyme may be found in the other 87%, although this raises the problem of how the enzyme comes into contact with a suitable substrate. As the enzyme activity was found in the soluble fraction on ultracentrifugation it is not in the cell granules. It may even be outside the cells. It may be on the post-synaptic membrane and may function in the degradation of neurotransmitters or in the excitation of the membrane.

It is also possible that the enzyme is ubiquitous and is not specifically important in neuronal functioning. 5MTHF dependent enzyme activity has been found (Table II) in lung, liver, and heart as well as brain tissue. The activity was greatest, in fact, in lung and heart. On the other hand, SAM dependent methyl transferase activity is also found in tissues outside the nervous system.

While enzyme, coenzyme, and substrate levels are all high in the caudate, this correspondence is not found elsewhere in the brain. 5MTHF is highest in the midbrain raphe (88) where enzyme activity is about half that found in the caudate. There is a large amount of enzyme activity in the cerebellum

whichhas minimal amounts of 5HT and DA and only small amounts of 5MTHF. Kemp and Powell (89-93) have extensively studied the structure of the caudate nucleus in the cat and conclude (93) that the caudate and cerebellum are similar in structure, connections, and function. They now seem to have a peculiar biochemical similarity. The cortex, hippocampus, and raphe have lower but significant enzyme activity.

Another approach to investigating the role of this enzyme would be to demonstrate its activity in vivo and to determine the action of its product. Epinine is not known to exist in vivo except in the parotid gland of a South American toad, Bufo marinus (10). In preliminary studies, C^{14}-5MTHF was injected into the tail vein of rats with the intention of assaying for radioactive catecholamine metabolites in the brain. After the injection of 0.5 to 3 μCi of C^{14}-5MTHF however, only background radioactivity was found in brain homogenates up to one hour after the injection. This contradicts the findings of Levitt (39), but the number of trials was too small for this to be conclusive evidence. C^{14}-5MTHF was also injected directly into the lateral ventricles using an interventricular cannula technique described by Verster et al (94). The ventricular injections consistently produced massive convulsions in the rats. The seizures may have resulted from the nonphysiologic pH (8.4), the ascorbic

acid in the carrier, or they may have been a demonstration of the seizure inducing property of high concentrations of folic acid. Brain homogenates were assayed as described and no epinine was found to be produced up to one hour after the interventricular injections were performed.

The 5MTHF dependent DA-N-methyl transferase may be important in the neuropsychiatric symptoms found in folate-B_{12} deficiencies. It is not known whether or not B_{12} is required for the methylation of dopamine as it is for homocysteine. If B_{12} is necessary, this system may malfunction in deficiency states. If B_{12} is not necessary then the reaction is unique and may be similar to that found in bacteria and lower animals.

The involvement of folate in methionine metabolism is interesting in light of the importance of SAM in catecholamine metabolism. Folate deficiencies may cause neuropsychiatric symptoms because of their effect on methionine rather than epinine or other product, and similarly abnormal methionine levels may affect folate.

Laduron has speculated that excess 5MTHF may lead to excess production of epinine which may function as a neurotransmitter and be involved in schizophrenia. The high concentration of the enzyme in the caudate makes this idea more plausible. The results in Figure 9 show that as the concentration of 5MTHF is increased the amount of epinine

produced increases linearly until a saturation point is reached. Depending on physiological conditions, this may occur *in vivo*. Except for deficiency states, however, it is rare *in vivo* for a coenzyme to so greatly affect the rate of a reaction.

The enzyme activity in the human caudate samples was very close to that found in the rat caudate. The presence of the enzyme in human brain means that there is a real possibility that it is important in human neuronal metabolism.

Wise and Stein (95) have found decreased levels of dopamine-B-hydroxylase in the post mortem brains of schizophrenics as compared to the brains of normal controls. Now that an enzyme which can N-methylate DA has been found in human caudate, it is possible that if dopamine-B-hydroxylase is decreased, DA may be methylated to epinine instead of hydroxylated to noradrenaline.

Bibliography

1. Thudichum,J.L.W.: A Treatise on the Chemical Constitution of the Brain, Bailliere, Tindall, and Cox, London, 1884.

2. Osmond, H., and Smythies, J.: Schizophrenia: A New Approach, J. Ment. Sci. 98:309-315, 1952.

3. Laduron, P.: N-Methylation of Dopamine to Epinine in Tissue using N-Methyl tetrahydrofolic acid as the Methyl Donor, Nature New Biology, 238:212-213, 1972.

4. Smythies, J.R.: Introduction to Amines and Schizophrenia, ed. by Himwich, H.E.,Kety,S.S., and Smythies,J.R., Pergamon Press, N.Y., 1967.

5. Axelrod, J.: O-Methylation of Epinephrine and Other Catechols in vitro and in vivo, Science, 126:400-401, 1957.

6. Axelrod, J., and Tomchick, R.: Enzymatic O-Methylation of Epinephrine and Other Catechols, J. Biol. Chem., 233:702-705.

7. Axelrod, J.: The Formation and Metabolism of Physiologically Active Compounds by N- and O- Methyltransferases,in Transmethylation and Methionine Biosynthesis, ed. by Shapiro,S.K., and Schenk,F., U. of Chicago Press, Chicago, pp.71-83, 1965.

8. Axelrod,J.: Enzymatic Formation of Psychotomimetic Metabolites from Normally Occurring Compounds, Science 134:343, 1961.

9. Axelrod, J.: The Enzymatic N-Methylation of Serotonin and Other Amines, J. Pharmac. Exp. Ther., 138:28-33, 1962.

10. Axelrod, J.: Purification and Properties of Phenylethanolamine-N-Methyl transferase, J. Biol. Chem. 237:1657-60, 1962.

11. Mandell,A. and Morgan, M.: Indole(ethyl)amine N-Methyltransferase in Human Brain, Nature New Biology, 230: 85-87, 1971.

12. Saavedra, J.M., and Axelrod,J.: Psychotomimetic N-methylated tryptamines: formation in brain in vivo and in vitro, Science 172:1365-1366, 1972.

13. Molinoff, P., and Axelrod,J.: Biochemistry of Catecholamines, J.Ann. Rev. of Biochem. 40:465-500,1971.

14. Bridgers,W.F., and Kaufman,S.: The Enzymatic Conversion of Epinine to Epinephrine, J. Biol. Chem. 237:526-528, 1962.

15. Kety,S.: Current Biochemical Approaches to Schizophrenia, NEJM, 276:325-331, 1967.

16. Boulton,A.: Biochemical Research in Schizophrenia, Nature, 231: 22-28, 1971.

17. Narasimhachari,N., Plaut,J.M., and Himwich,H.E.: Indolethylamine-N-Methyltransferase in Serum Samples of Schizophrenics and Normal Controls, Life Sci. Vol. 11 Pt. II, pp. 221-227, 1972.

18. Wyatt,R.J., Saavedra,J.M., and Axelrod,J.: A Dimethyltryptamine-Forming Enzyme in Human Blood, Am. J. Psych. 130: 754-601, 1973.

19. Hoffer,A., Osmond,J.,Callbeck,M.J., and Kahun,I.: Treatment of Schizophrenia with nicotinic acid and nicotinamide, J. Clin. & Exp Psychopath, 18: 131-158, 1957.

20. Hoffer,A., and Osmond,J.: Treatment of Schizophrenia with nicotinic acid, Acta Psychiat. Scand. 40:171-189,1964.

21. Pollin,W.,Cardon,P., and Kety,S.: Effects of Amino Acid Feedings in Schizophrenic Patients Treated with Iproniazid, Science, 133:104-105, 1961.

22. Brune,G.G., and Himwich,H.E.: Biogenic Amines and Behavior in Schizophrenic Patients in Recent Advances in Biological Psychiatry, Vol. 5, ed. by Wortis, Plenum Press, pp. 144-160, 1963.

23. Alexander,F.,Curtis,G.C., Sprince,H., and Crosley,A.P.: L-methionine and L-tryptophan feedings in non-psychotic and schizophrenic patients with and without tranylcypromine, J. Nerv. Ment. Dis. 137:135-142,1963.

24. Friedhoff, A.J., and Van Winkle,E. : Isolation and Characterization of a Compound from the urine of Schizophrenics, Nature, 194:897-898,1962.

25. Siegel,M., and Tefft,J.: Pink spot and its components in Normal and Schizophrenic urine, J. Nerv. Ment. Dis. 152: 412-426,1971.

26. Stabenau,J.R., Creveling,C.R., and Daly,J.: The 'pink spot', 3-4-dimethoxyphenylethylamine, common tea, and schizophrenia, Am. J. Psychiat. 127:611, 1970.

27. Narasimhachari,N.,Plaut,J., and Himwich,H.: 3,4 Dimethoxyphenylethylamine, A normal or abnormal metabolite, J. Psychiat. Res., 9:325-328, 1972.

28. Israelstam,D.M., Sargent,T.,Finley, N.N.,Winchell,H.S., Fish,M.B., Motto,J., Pollycove,M., and Johnson,A.: Abnormal methionine metabolism in schizophrenia and depressive states: A preliminary report, J. Psychiat, Res., 7:185,1970.

29. Carey, M.C., Donovan, D. E., Fitzgerald,O., and McCauley, F.D.: Homocystinuria-I. A Clinical and Pathological Study of Nine subjects in six families, Am. J. Med. 45:7, 1967.

30. Laduron,P.: N-Methylation of Dopamine to Epinine in Adrenal Medulla: A New Model for the Biosynthesis of Adrenaline, Arch. Int. Pharmacodyn. 195:197-208, 1972.

31. Laduron,P.: N-Methyldopamine (epinine), A precursor of Adrenaline in the Adrenal Medulla, Arch. Int. Pharmacodyn. Ther. 196 Suppl. 196:304-306, 1972.

32. Esplin,Don: Centrally Acting Muscle Relaxants, Drugs for Parkinson's Disease, in The Pharmacological Basis of Therapeutics, ed. by Goodman and Gillman, Macmillan Co., N.Y., pp. 226-236,1970.

33. Barger,G. and Dale, H.H.: Chemical Structure and Sympathomimetic Action of Amines, J. Physiol., 41:19-59,1910.

34. Banerjee,S., and Snyder,S.: Methyltetrahydrofolic Acid Mediates N- and O- Methylation of Biogenic Amines, Science, 182:74-75, 1973.

35. Hoffbrand,A.V., and Peters,T.J.: Recent Advances in knowledge of clinical and biochemical aspects of folate, Schweiz.med. Wschr. 100:1954-60, 1970.

36. Herbert,V.: Drugs Effective in Megaloblastic Anemias: Vitamin B_{12} and Folic Acid, in The Pharmacological Basis of Therapeutics, ed. by Goodman and Gillman, Macmillan Co., pp. 1414-1444, 1970.

37. Herbert,V., and Zalusky,R.: Selective concentration of folic acid activity in cerebrospinal fluid, Fed. Proc. 20: 453, 1961.

38. Allen,C.D., and Klipstein,F.A.: Brain folate concentration in rats receiving diphenylhydantoin, Neurology, 20:403, 1970.

39. Levitt,M., Nixon,P., Pincus, J., and Bertino,J., Transport Characteristics of Folates in Cerebrospinal Fluid: a Study Utilizing Doubly Labeled 5-Methyltetrahydrofolate and 5-Formyltetrahydrofolate, J. Clin. Inves. 50:1301-1308,1971.

40. Blakely, R.L., The Biochemistry of Folic Acid and Related Pteridines, Frontiers of Biology Vol. 13, North-Holland Pub. Co., London, 1969.

41. Mangum,J.H., and North,J.A.: Isolation of a Cobalamin Containing 5-Methyltetrahydrofolate-Homocysteine Transmethylase from Mammalian Kidney, Biochem. 10:3765-3769,1971.

42. Mahler,J.R., and Cordes,E.H.: Biological Chemistry, Harper and Row,N.Y.,1966.

43. Reynolds,E.H.: Anticonvulsant Drugs,Folic Acid Metabolism, Fit Frequency, and Psychiatric Illness, Psychiat. Neurol. Neurochir., 74:167-174, 1971.

44. Reynolds,E.H.,Preece,J., and Johnson,A.C.: Folate Metabolism in Epileptic and Psychiatric Patients, J. Neurol. Neurosurg. Psychiat. 34:726-732, 1971.

45. Rothfeld, P., The relationship of Folic Acid Deficiency to Neurologic and Psychiatric Disorders, Yale Medical Sch. M.D. Thesis, 1972.

46. Snaith,R.P., Mahta,S., and Raby,A.H.: Serum Folate and Vitamin B_{12} in Epileptics with and without Mental Illness, Brit, J. Psych. 116:179, 1970.

47. Callaghan,N., Mitchell,R., and Cotten,P.: The relationship of serum folic acid and Vitamin B_{12} levels to psychosis in epilepsy, Irish J. med. sci. 2:497, 1969.

48. Reynolds, E.H., Preece,J., and Chanarin,I., Folic Acid and Anticonvulsants, Lancet I:1264-65, 1969.

49. Preece,J., Reynolds,E.H., and Johnson,A., Relation of Serum to Red Cell Folate Concentrations in Drug Treated Epileptic Patients, Epilepsia, 12:335-340, 1971.

50. Reynolds,E.H., Gallagher,B.B., Mattson,R.H., Bowers,M., Johnson,A.L.: Relationship between Serum and Cerebrospinal Fluid Folate, Nature, 240: 155-57, 1972.

51. Reynolds,E.H., Mattson, R.H., Gallagher, B.B.: Relationships between serum and cerebrospinal fluid anti-convulsant drug and folic acid concentrations in epileptic patients, Neuro., 22:841-844, 1972.

52. Horvitz,S.S., Klipstein,F.A., and Lovelace, R.E.: Relationship of abnormal folate metabolism to neuropathy developing during anticonvulsant therapy, Lancet I:563-565,1968.

53. Reynolds, E.H., Chanarin,I, Milner, G., and Matthews, P. M.: Anticonvulsant Therapy, Folic Acid, and Vitamin B_{12} Metabolism and Mental Symptoms, Epilepsia 7:261-270, 1966.

54. Neubauer,C.: Mental Deterioration in Epilepsy Due to Folate Deficiency, Brit. Med. J., 2:759-761, 1970.

55. Spaans,F.: No Effect of Folic Acid Supplement on CSF Folate and Serum Vitamin B_{12} in Patients on Anticonvulsants, Epilepsia, 11:403-411, 1970.

56. Mattson,R., Gallagher,B., Reynolds,E.H., and Glass,D.: Folate Therapy in Epilepsy: A Controlled Study, Arch. Neurol. 29:78-81, 1973.

57. Reynolds,E.H., Rothfeld,P., Pincus,J.H.: Neurological Disease associated with Folate Deficiency, Br. Med. J. 2:398-400, 1973.

58. Pincus,J.H., Reynolds,E.H., and Glaser,G.: Subacute Combined System Degeneration with Folate Deficiency, JAMA, 221:496-7, 1972.

59. Strachan,R., and Henderson,J.: Dementia and Folate Deficiency, Quart.J. Med. 36:189-203, 1967.

60. Carney,M.W.P., and Sheffield,B.F.: Associations of subnormal serum folate and vitamin B_{12} values and effects of replacement therapy, J. Nerv. Ment. Dis. 150:404-412, 1970.

61. Herbert,V.: Inborn Errors in Folate Metabolism-A Cause of Mental Retardation?, Ann. Int. Med. 68:956-7, 1968.

62. Hunter, R., Barnes, J., Oakely, H.F., and Matthews, D.M.: Toxicity of Folic Acid Given in Pharmacological Doses to Healthy Volunteers, Lancet, I:61-63, 1970.

63. Hunter,R., and Barnes,J.: Toxicity of Folic Acid, Lancet I:755, 1971.

64. Hellstrom,L.: Lack of Toxicity of Folic Acid Given in Pharmacological Doses to Healthy Volunteers,Lance I:59-61, 1971.

65. Reynolds, E.H.: Anticonvolsants, Folic Acid, & Epilepsy, Lancet II:1376-1378, 1973.

66. Mayersdorf,A., Strelff, R.R., Wilder, B.J., Hammer, R.H., Folic Acid and Vitamin B_{12} Alterations in Primary and Secondary Epileptic Foci, Neurol. 21:418, 1971.

67. Klawans, H., Goety,C., and Westheimer,R.: Pathophysiology of Schizophrenia and the Striatum, Dis. Nerv. Sys. 33: 711-719, 1972.

68. Dahlström,A., and Fuxe,K.: Evidence for the existence of monoamine-containing neurons in the central nervous system, Acta Physiol. Scand. 62 syppl 232:1-55, 1965.

69. Fuxe,K.: Evidence for the existence of monoamine neurons in the central nervous system IV-Distribution of monoamine nerve terminals in the central nervous system, Acta. Physiol. Scand. 64 suppl 247:41-85, 1965.

70. Anden,N.-E., Carlsson,A., Dahlström,A., Fuce,K.,Hillarp,N.-A., and Larsson,K.: Demonstration and mapping out of nigro-striatal dopamine neurons, Life Sci. Oxford 3:523-530, 1964.

71. Anden,N.-E.,Dahlström,A., Fuxe,K., and Larsson,K.: Further evidence for the presence of nigro-neostriatal sopamine neurons in the rat, Am.J. Anat. 116:329-333,1965.

72. Anden,N.-E., Dahlström,A., Fuxe,K., Larsson,K., Olson,L., and Ungerstedt, U.: Ascending monoamine neurons to the telencephalon and diencephalon, Acta Physiol, Scand, 67:313-326, 1966.

73. Aghajanian, G.K.: Serotonin-Containing Neurons in the Central Nervous System, in Neurotransmitters Res. Publ. A.R.N.M.D., Vol. 50, pp. 181-192,1972.

74. Ungerstedt, U.: Stereotaxic Mapping of the Monoamine Pathways in the Rat Brain, Acta Physiol. Scand. Suppl 367:1-48, 1971

75. Hokfelt,T., and Ungerstedt,U.: Electron and Fluorescence Microscopical Studies on the Nucleus Caudatus Putamen of the Rat after Unilateral Lesions ofAscending Nigro-Neostriatal Dopamine Neurons, Acta Physiol. Scand. 76: 415-426, 1969.

76. Kuhar, M.J. and Aghajanian,G.K.: Selective accumulation of H^3 serotonin by nerve terminal of raphe neurons: an autoradiographic study, Nature, 41:187-189, 1973.

77. Heller,A., and Moore,R.Y.: Effect of Central nervous system lesions on brain monoamines in the rat, J. Pharmacol. Exp. Ther. 150:1-9, 1965.

78. Iverson,L.L., and Uretsky,N.J.: Biochemical effects of 6-hydroxydopamine on catecholamine containing neurons in the rat central nervous system, in 6-Hydroxydopamine and Catecholamine Neurons, ed. T. Malmfors and H. Thoenen, North-Holland, London, pp. 171-186,1971.

79. Bedard,P., Larochelle,L., Parent,P., and Poirer,L.J.: The nigrostriatal pathway: A correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey, Exp. Neurol. 25:365-377, 1969.

80. Connor,J.D.: Caudate Nucleus Neurons: Correlation of the Effects of Substantia Nigra Stimulation with Iontophoretic Dopamine, J. Physiol. 208:691-703, 1970.

81. Walters,J.: Dopaminergic Neurons: Effect of Gamma-Hydroxybutyrate, Yale University Dept. of Pharm. Phd. Thesis, 1972.

82. Konig, J.F.R., and Klippel, R.A.: The Rat Brain, A Stereotaxic Atlas, Williams and Wilkins, Baltimore, 1963.

83. Valzelliland,L., and Garattini,S.: Biogenic amines in discrete brain areas after treatment with monoamineoxidase inhibitors, J. Neurochm. 15:259-261,1968.

84. Kuhar,M.J.,Roth,R.H., and Aghajanian,G.K., Selective reduction of tryptophan hydroxylase activity in rat forebrain after midbrain raphe lesion, Brain Research, 35:167-176, 1971.

85. Roth,R.H., Stjarne,L., Levine, and Giarman,N., Abnormal Regulation of Catecholamine Synthesis in Pheochromocytoma, J. Lab. Clin. Med. 72:397-403,1968.

86. Stjarne,L.,Lishajko,F.: Localization of the different steps in noradrenaline synthesis to different fractions of bovine splenic nerve homogenate, Biochem. Pharmacol. 16: 1723, 1967.

87. Lowry,O., Rosebrough,N., Fan,A., and Randall,R., Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. 193:265, 1951.

88. Korevaar,W.C., Geyer, M.,Knapp,S., Hsu,L., and Mandell,A.: Regional Distribution of 5 Methyl Tetrahydrofolic Acid in Brain, Nature New Biology 245:244-245, 1973.

89. Kemp,J.M.,and Powell, T.P.S.: The Structure of the Caudate Nucleus of the Cat: Light and Electron Microscopy, Phil. Trans. R. Soc. Lond. B. 262:383-401.

90. Kemp, J.M., and Powell, T.P.S.: The Synaptic Organization of the Caudate Nucleus, Phil. Trans. R. Soc. Lond. B. 262:403-412,1971.

91. Kemp, J.M., and Powell, T.P.S.: The Site of Termination of Afferent Fibres in the Caudate Nucleus, Phil. Trans. R. Soc. Lond. B. 262:413-427, 1971.

92. Kemp, J.M., and Powell, T.P.S.: The Termination of Fibres From the Cerebral Cortex and Thalamus upon the Dendritic Spines in the Caudate Nucleus: A Study with the Golgi Method, Phil. Trans. R. Soc. Lond. B.262:429-439, 1971.

93. Kemp,J.M.,and Powell, T.P.S.: The Connexions of the Striatum and Globus Pallidus: Synthesis and Speculation, Phil. Trans. R. Soc. Lond. B. 262:441-457, 1971.

94. Verster, F., Robinson, C., Hengweld, C., and Bush,E.: Freehand Cerebroventricular Injection Technique for Unanesthetized Rats, Life Sci. Vol 10 Pt. I pp. 139-1402, 1971.

95. Wise, C.D., and Stein,L., Dopamine-β-Hydroxylase Deficits in the Brains of Schizophrenic Patients, Science 181:344-347, 1973.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copted without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS DATE